Potential fungicide activity of endophytic fungi isolated from ginger against *Botrytis cinerea*

Rogelio Borrego López[1] , Alejandro Bódalo Ponce[1] , Victoria Eugenia González Rodríguez[1] , Carlos Garrido Crespo[1] , María Carbú Espinosa de los Monteros[1] , María Dolores Vela-Delgado[2] , Hernando José Bolívar Anillo[3] , M. Teresa Gutierrez[1], Jesús M. Cantoral Fernández[1]

[1] Departamento de Biomedicina, Biotecnología y Salud Pública, Laboratorio de Microbiología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, 11510, Puerto Real, Spain.

[2] IFAPA Rancho de la Merced, Sede CHIPIONA., Camino Esparragosa s/n. 11550, Chipiona, Spain

[3] Programa de Microbiología, Facultad de Ciencias Básicas y Biomédicas, Universidad Simón Bolívar, Barranquilla, Colombia.

E-mail: rogelio.borrego@uca.es

Introduction

Botrytis cinerea is a phytopathogenic fungus that causes gray mould disease and infects more than 1,400 species of cultivated and wild plants, being one of the main responsible for the increasing costs on phytosanitary treatments as well as significant economic losses in the agricultural sector. Chemical fungicides are the main and most effective method to date to fight gray mould, however, due to their harmful effects, it is necessary to look for another, less harmful method, such as biological control. Endophytic microorganisms, which are those that live inside plants establishing a symbiotic relationship, are an important source of new biofungicides with the potential to exert biocontrol of *Botrytis cinerea*. Due to its medicinal characteristics and its capacity to host microorganisms potentially applicable to biotechnology, in this study the endophytic fungi of ginger (*Zingiber officinale*) were investigated in order to find a candidate for a biocontrol agent.

Material and Methods

- **Isolation**

Fungal endophytes were obtained from the inside of ginger clean and free of ground debris tubers. Tubers were previously superficially sterilized as indicated in Bolívar-Anillo *et al.* 2020. After that, the ginger was peeled and the rest of each tuber was macerated with 0.9% NaCl. 100 µL the resulting liquid was spread over a PDA plate. Individual colonies were reseeded onto differents PDA plates until each fungus was completely isolated.

- **Morphological and molecular identification of fungi**

Isolated fungi were observed under microscope to distinguish their spores and conidia ir order to determine their taxonomical family. Next, we made DNA extractions for each fungus following the method described by Bolívar-Anillo *et al.* 2020 with some modifications. DNA was amplified by PCR using primers pairs ITS1-ITS4 (White et al., 1990), LROR-LR7 (Cubeta et al., 1991) and Bt2a-Bt2b (Glass & Donaldson, 1995). PCR products were purified and sequenced. The data were compared to those registered in BLAST.

- ***B. cinerea* against edophytic fungi**

Nine milimeter media discs with *B. cinerea B05.10* micelium and each endophytic fungus were put on PDA plates on opposite sides and they were incubating for 7 days at 25ºC with continuous lighting. Once the fungi had grown we measured the area of each mycelium to determine the percentage of inhibition in comparison with a *B. cinerea* control.

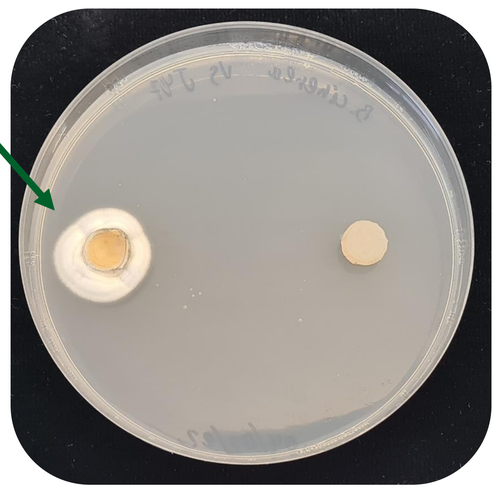
Results y and discussion

We isolated 9 different species of fungi from ginger tubers identified as: *Aspergillus ustus*, *Aspergillus keveii, Penicillium chrysogenum*, *Penicillium steckii*, *Penicillium melinii*, *Aspergillus sp.*, *Penicillium citroenigrum*, *Pseudogymnoascus* (*Geomyces*) *pannorum* and *Plectosphaerella cucumerina.* In the next table results of inhibition are shown.

Fungus	% Radial inhibition (Per area)
Plectosphaerella cucumerina	49,20
Aspergillus ustus	80,06
Aspergillus keveii	32,21
Penicillium chrysogenum	70,72
Penicillium steckii	50,66
Penicillium melinii	98,56
Aspergillus sp.	0,00
Penicillium citroenigrum	51,94
Pseudogymnoascus (Geomyces) pannorum	33,66

In the light of the results, the top three inhibitori fungi were *Penicillium melinii* (98.56%), *Aspergillus ustus* (80.06%) and *Penicillium chrysogenum* (70.72%). However, all of the fungi except *Aspergillus sp.* showed inhibitory activity, so it would be interesting to study the rest of them under different conditions so we can determine how to increase the power of inhibition of each fungus.

Moreover, it could be interesting to ferment each specie and obtain their extracts to study if they have inhibitory effect against *B. cinerea*. In case of positive results, the extracts could be separated by HPLC and the compounds contributing to the inhibitory activity could be identified. That compound could be used as precursor of new biological antifungal products.



References

Bailey, J. A. & Jeger, M. J. (1992). Colletotrichum: biology, pathology and control. CBA International. Wallingford, UK

Cubeta, M. A., Echandi, E., Abernethy, T., & Vilgalys, R. (1991). Characterization of Anastomosis Groups of Bionucleate Rhizoctonia Species Using Restriction Analysis of an Amplified Ribosomal RNA Gene. Phytopathology, 81, 1395–1400.

Botrytis – The fungus, the pathogen and its management in agricultural systems, eds. S. Fillinger and Y. Elad, (2016). Springer International Publishing, pp. 413-4864.

Glass, N. L., & Donaldson, G. C. (1995). Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology, 61, 1323–1330.

EU Pesticides database (2015). http://ec.europa.eu/food/plant/pesticides/eupesticides-database/public/event

H. J. Bolívar Anillo, “Evaluación de la capacidad antifúngica y de promoción de crecimiento vegetal de la microbiota endófita aisladas de plantas de maíz (Zea mays) así como de hongos antagonistas aislados de otras fuentes,” Universidad de Cádiz, 2018

O’Connell RJ, Thon MR, Hacquard S, et al (2012). Lifestyle transitions in plant pathogenic Colletotrichum fungi deciphered by genome and transcriptome analyses. Nat Genet 44:1060–1065.

White, T. J., Bruns, T., Lee, S., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In PCR Protocols (pp. 315–322). Elsevier.

Acknowledgments

This project has been financed by the FEDER (European Regional Development Fund, Ministry of Economy, Knowledge, Business and University), Junta de Andalucía and by the University of Cádiz.

Unión Europea
Fondo Europeo de Desarrollo Regional “Una manera de hacer Europa”

Junta de Andalucía
Consejería de Economía, Conocimiento, Empresas y Universidad